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STUDIES ON THE GERMINATION OF CONIDIA AND THE SPORULATION OF *CERCOSPORA CRUENTA* SACC.

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ABSTRACT - The effects of temperature, relative humidity and light regimes on the sporulation and germination of conidia of *Cercospora cruenta* were carried out. The effects of light intensity, brestan and benlate on conidial germination were also investigated. Optimum germination and sporulation were recorded between 25-30°C at relative humidity of 87.5-100%. Heaviest sporulation in culture and on naturally infected leaves occurred in continuous darkness. A gradual decrease in conidial germination was recorded with increase in light intensity, while brestan was more effective in the conidial inhibition than benlate.

RESUME - Étude des effets de la température, de l'humidité relative et des régimes de lumière sur la sporulation et la germination de conidies de *Cercospora cruenta*, et recherche des effets de l'intensité lumineuse, du brestan et du benlate sur la germination des conidies. Les conditions de germination et de sporulations optimales sont 25-30°C avec 87,5-100% RH. Une meilleure sporulation, en culture et sur feuilles naturellement infectées, est obtenue à l'obscurité. On enregistre une diminution graduelle de la germination des conidies à mesure que la lumière s'intensifie, et on note que le brestan a plus d'effet que le benlate sur l'inhibition de germination des conidies.

KEY WORDS : conidial germination, sporulation, *Cercospora cruenta*.

INTRODUCTION

Conflicting reports have been presented by several workers on the effect of light regimes on the sporulation of *Cercospora* spp. Sporulation in *C. nicotianae* (Stavely & Nimmo, 1969) and *C. zebrina* (Berger & Hanson, 1963) was heavier in continuous light while *C. beticola* (Fajola, 1971) sporulated heaviest in continuous darkness. However, numerous conidia are often found on *Cercospora* infected leaves in the field during the early hours in the morning, an indication that the conidiation takes place more at night.

The most critical stage however, in the life of an infecting fungus, is the successful spore germination and initial penetration of the host. The infection of the plant by such a fungus depends on at least, some extension of

its germ tube. At this time, the fungus is most dependent upon and susceptible to influence of the physical, biochemical and biological environment. The optimum temperature for conidial germination of *Cercospora arachidicola* was 20-30°C and 37°C was lethal (Oso, 1972). Latch & Hanson (1962) recorded 100% spore germination for *C. davisii* at 20-28°C. Emua (1980) reported that sporulation and germination of conidia of *C. apii* and *C. contraria* were greatly enhanced by high relative humidity.

Previous studies on spore germination of the *Cercospora* spp. have shown the production of secondary conidia: *C. bougainvilleae* (Sobers & Martinez, 1966) and *C. arachidicola* (Oso, 1972) while secondary conidiophore and conidia were found in *C. zebrina* (Berger & Hanson, 1963). Fajola (1978) reported that the conidiophores and conidia of five *Cercospora* spp. increased in length and in septation with increase in relative humidity, or temperature up to 25°C.

These studies have provided useful informations on the effects of some environmental factors on the reproductive structures of *Cercospora*. However, most species of *Cercospora* studied have not been removed from their immediate environment (the host). This investigation is based on *in vitro* and *"in vivo"* studies carried out on the effects of 3 environmental factors - light, temperature and relative humidity (RH) - on the germination and sporulation of *Cercospora cruenta*. The effectiveness of 2 fungicides was also tested against its conidial germination.

MATERIALS AND METHODS

The investigation carried out was on the organism, *Cercospora cruenta* Sacc., that causes severe leaf spotting of *Vigna unguiculata* (Savi ex Haask) Walp. Infected leaves of *V. unguiculata* were harvested from the experimental farms at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria between 8 a.m. - 10 a.m. (07-9h GMT).

A preliminary experiment on the effect of conidial concentration on germination was carried out and a concentration of conidia at 16.10^4 conidia/ml gave the highest germination level. Therefore a spore load of 15.10^4 conidia/ml was used for germination experiments.

The conidial germination on glass slide, cowpea leaf decoction nutrient agar and host epidermal strip were carried out. A spore load suspension was placed on glass slide to form a film while another spore load suspension was placed on each of 4 zones of Petri dish. A teased epidermal strip from healthy host leaf was obtained, placed on a glass slide and a drop of spore load suspension was deposited on the strip. Each inoculated substrate was placed in a desiccator with about 95% RH at 28.5°C, incubated for 16h and observations on the rate of germination were made every 1h.

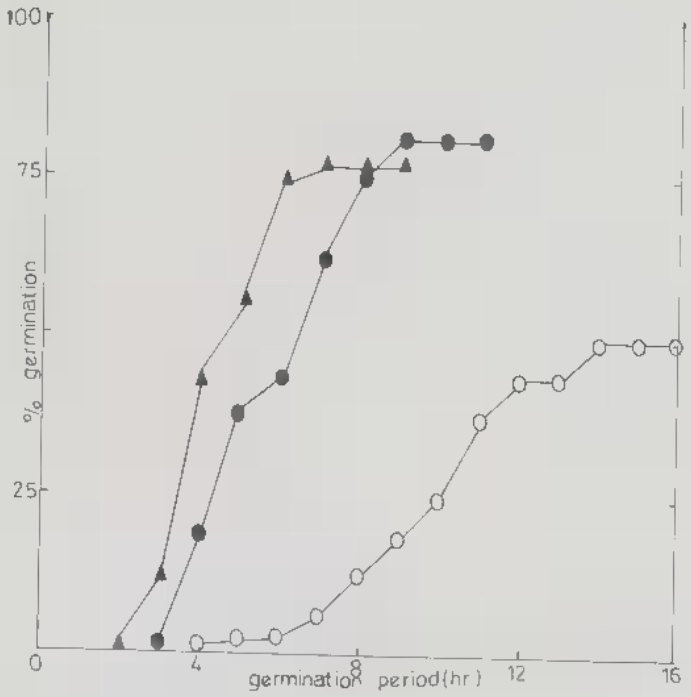

Fig. 1 - Percentage germination of conidia of *C. cruenta* on 3 substrates: glass slide (0--0), host epidermal strip (●--●), *Vigna unguiculata* leaf decoction nutrient agar (▲--▲) incubated for 16h at 28.5°C under about 95% RH (results are means of 5 replicates).

Fig. 1 - Germination (%) des conidies de *Cercospora cruenta* sur 3 substrats: lame de verre (0--0), épiderme de l'hôte (●--●), gélose nutritive à base de décoction de feuilles de *Vigna unguiculata* (▲--▲). Incubation 16h à 28,5°C et 95% RH (moyennes de 5 exp.)

The induction of conidia in culture was done by culturing *C. cruenta* on cowpea leaf nutrient agar and incubated at 25°C for 72h. The estimation of sporulation was carried out by following the method of Stavely & Nimmo (1969). Conidial production was induced on naturally infected leaves by following the method of Nagel (1934). Diseased leaves were washed and blotted dry with filter paper, placed in high humidity chambers (95%) for 24 h for the production of new crop of conidia. Estimation of sporulation was then carried out following the method of Chee (1976). Ten 4mm discs of culture or 5 pieces of diseased leaves (0.5cm by 0.5cm) were placed in 10ml distilled water, shaken on a mechanical shaker for 1 min to dislodge the conidia and suspension was filtered through muslin. The spore load of the conidial suspension was then estimated using a Hawksley Cristallite B.S. 748 haemocytometer as described by Purvis & al. (1966). The effects of light,

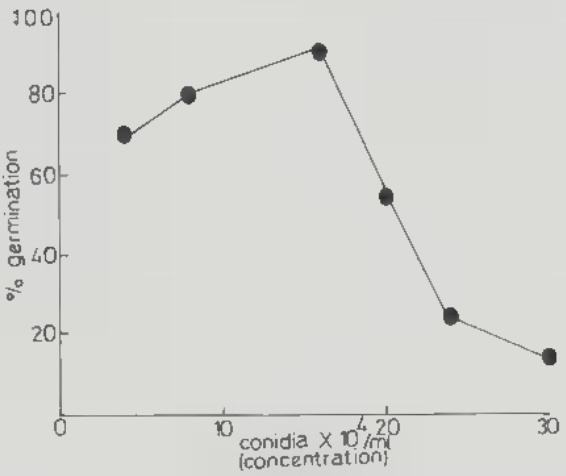

Fig. 2 - Effet of spore concentration on the germination of conidia of *Cercospora cruenta* on glass slide incubated for 6h at 28.5°C under about 95% RH (results are means of 5 replicates).

Fig 2 - Effet de la concentration des spores sur la germination des conidies de *C. cruenta* sur lame de verre. Incubation 6h à 28,5°C et 95% RH (moyennes de 5 exp.).

temperature and RH on the *in vitro* germination and on sporulation (*"in vivo"* and *in vitro*) were carried out. Experiments on temperature relations were conducted in incubators set at 5 to 40°C (5°C interval). Six levels of relative humidity: 0, 32.5, 52, 75, 87.5 and 100% (Winston & Bates, 1960) were obtained in desiccators.

The light regimes were made up of continuous light, continuous darkness, alternating light and dark. The continuous light was achieved when a growth chamber was fitted with four 40 watt 4 feet long Phillips fluorescent bulbs, 61cm above medium surface. The light intensity was 885 Lux on medium surface, measured with a highly sensitive LI-COR photometer. The light intensities if 100, 500, 750, 1000, 1600 and 2000 Lux were obtained by appropriately adjusting the height of the incubated culture or diseased leaf. A clean box completely wrapped with black opaque photographic paper was used to provide the continuous darkness. For the alternating periods of light and darkness, diseased leaves and inoculated cultures were maintained 12h in continuous light and 12h in continuous darkness except in germination experiments where the periods of alternating light/darkness was 3.5h. There were 5 determinations for each set of experiments.

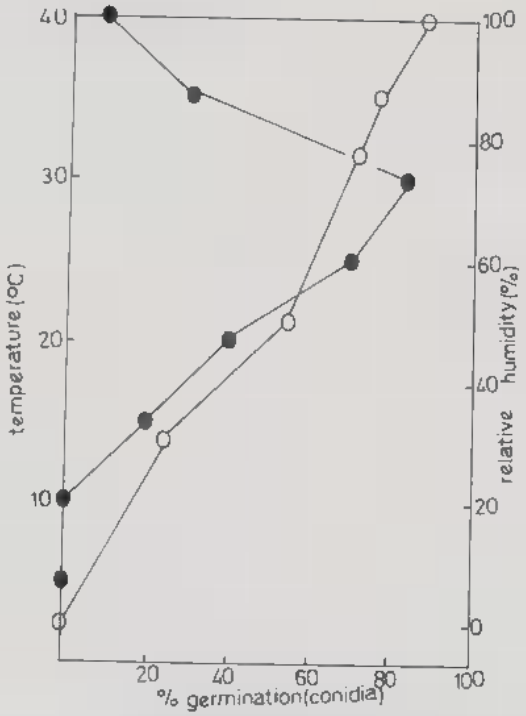

Fig. 3 - Effects of temperature and RH on the germination of conidia of *C. cruenta* on glass slide incubated for 24h (results are means of 5 replicates).

Fig. 3 - Effets de la température et de l'humidité relative sur la germination des conidies de *Cercospora cruenta* sur lame de verre. Incubation 24h (moyennes de 5 exp.).

RESULTS

Conidia accumulated on the spots from clustered conidiophores and were more conspicuous on the abaxial surface of leaflet. Conidium was brown to pale brown, straight to curved with a well defined basal hilum scar, 2-11 septate, measured 33.4-133.6μm x 3.3-10.0μm before incubation but measured 50.1-150.3μm x 3.3-13.4μm before germ tube emergence.

Germination of conidia on glass slide started after 5h, when about 3% germination was recorded while maximum germination (about 50%) was recorded between 14-16h of incubation (Fig. 1). Conidial germination on cowpea leaf decoction nutrient agar was observed after 3h when about 12.5% germination was recorded (Fig. 1) while about 75% germination was recorded between 7-9h of incubation. Germination of conidia on host epidermal strip occurred after 4h when about 18.7% germination was obtained

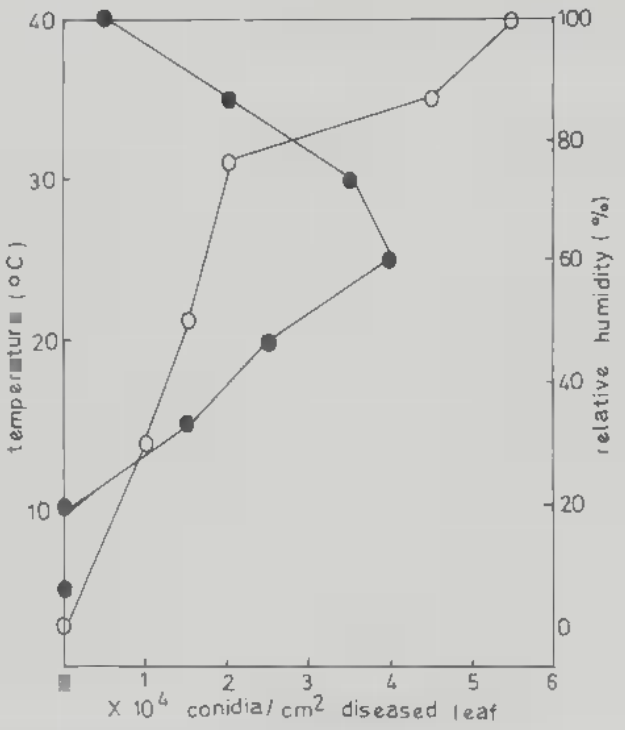

Fig. 4 - Effects of temperature and RH on the sporulation of *Cercospora cruenta* on naturally infected cowpea leaf incubated for 24h (results are means of 5 replicates).

Fig. 4 - Effets de la température et de l'humidité relative sur la sporulation de *C. cruenta*, sur feuille de *Vigna unguiculata* naturellement infectée. Incubation 24h (moyennes de 5 exp.).

and about 81% germination was recorded after 9h (Fig. 1). The number of germ tubes produced was maximum in culture and ranged from 2-10.

The effect of spore concentration on conidial germination showed that there was an increase in germination with increase in spore concentration where 70, 80 and 90% germination were recorded at 4.10^4 spore/ml, 8.10^4 spore/ml and 16.10^4 spore/ml respectively (Fig. 2). With high concentrations, there were a decline in % germination.

The effects of temperature and relative humidity on conidial germination are summarised in Fig. 3. There was no germination at 5-10°C while increase in percentage germination was recorded between 15-35°C, with optimum at 30°C. About 25% germination was recorded at 32.5% RH while about 75 and 85% germination were recorded at 87.5 and 100% RH respectively. No germination was recorded at 0% RH.

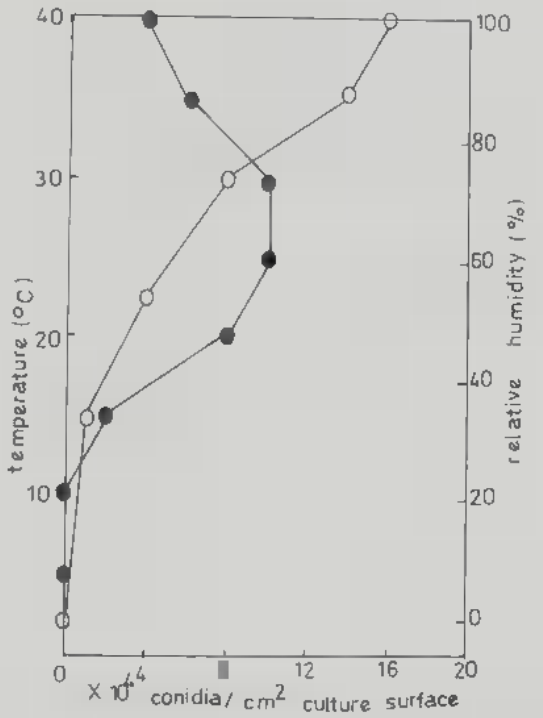

Fig. 5 - Effects of temperature and RH on the sporulation of *C. cruenta* cultured on cowpea leaf decoction nutrient agar incubated for 24h (results are means of 5 replicates).

Fig. 5 - Effets de la température et de l'humidité relative sur la sporulation de *Cercospora cruenta*, sur gélose nutritive à base de décoction de feuilles de *Vigna unguiculata*. Incubation 24h (moyennes de 5 exp.).

Observations on the effects of temperature and relative humidity on sporulation on diseased leaves and in culture are summarised in Fig. 4 and 5. Sporulation occurred between 15-40°C both on naturally infected leaves and in culture, with optimum at 25°C (Fig. 4) on diseased leaves and 25-30°C in culture (Fig. 5). Sporulation at 40°C was poor on diseased leaves while it was significant in culture. However, the results obtained from the effect of RH on sporulation showed that it has a greater effect on sporulation than temperature. No sporulation was recorded at levels below 32.5% RH, rather, sporulation increased with increase in RH.

The results of the experiments on the effect of light regimes on sporulation on naturally infected leaves and in culture are shown in Fig. 6 and 7. There was sporulation at all the light regimes. However, the highest sporulation was recorded in continuous darkness in both *"in vivo"* and *in vitro* conditions and least sporulation occurred in continuous light. The order of

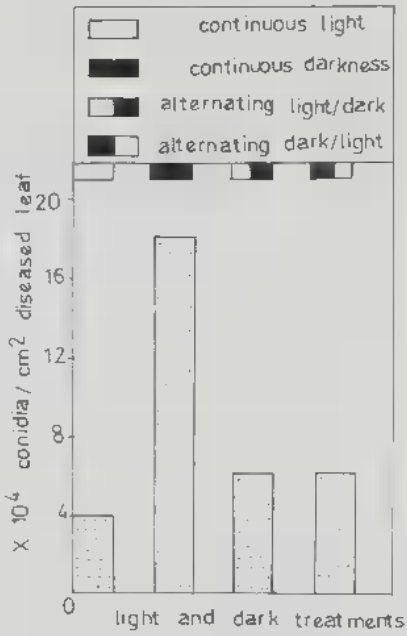

Fig 6 - Effect of light regimes on sporulation of *Cercospora cruenta* on naturally infected cowpea leaf incubated at 28.5°C for 24h (results are means of 5 replicates).

Fig. 6 - Effet des régimes de lumière sur la sporulation de *C. cruenta*, sur feuille de *Vigna unguiculata* naturellement infectée. Incubation 24h à 28,5°C (moyennes de 5 exp.).

Table 1: Effect of light intensity on the germination of conidia of *Cercospora cruenta* on cowpea leaf decoction nutrient agar incubated for 6h.

Tableau 1 - Effet de l'intensité de la lumière sur la germination des conidies de *C. cruenta*, sur gélose nutritive à base de décoction de feuilles de *V. unguiculata*. Incubation 6h.

light intensity (Lux)	recorded temperature (°C)	mean germination (%)
100	29	78.9 ± 1.6
500	29	80.7 ± 1.4
750	29	76.9 ± 2.0
1000	31	63.6 ± 1.0
1600	32	58.0 ± 1.2
2000	34	27.3 ± 0.5

Data are means of 5 replicates.

first exposure to either light or darkness had no effect on the rate of sporulation on diseased leaves but significant difference was recorded in culture during the period of alternating darkness and light (Fig. 7).

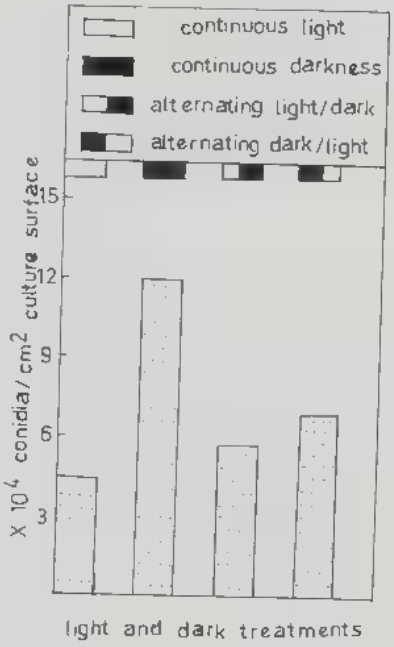

Fig. 7 - Effect of light regimes on the sporulation of *C. cruenta* cultured on cowpea leaf decoction nutrient agar incubated for 24 at 28.5°C (results are means of 5 replicates).

Fig. 7 - Effet des régimes de lumière sur la sporulation de *Cercospora cruenta*, sur gélose nutritive à base de décoction de feuilles de *Vigna unguiculata*. Incubation 24h à 28,5°C (moyennes de 5 exp.).

Table 2 - Effect of light regimes on the germination of conidia of *Cercospora cruenta* on cowpea leaf decoction nutrient agar incubated for 6h at 28.5°C.

Tableau 2 - Effet des régimes de lumière sur la germination des conidies de *C. cruenta*, sur gélose nutritive à base de décoction de feuilles de *V. unguiculata*. Incubation 6h à 28,5°C.

light regimes	% mean germination
continuous light (7h)	81.8 ± 1.3
continuous darkness (7h)	81.0 ± 1.1
3.5h light + 3.5h darkness	92.9 ± 1.4
3.5h darkness + 3.5h light	94.0 ± 1.2

Data are means of 5 replicates.

The observations on the effect of light intensity on conidial germination showed that there was a decrease in germination with increase in light intensity (Tab. 1). The results obtained on the effect of light regimes on ger-

mination however, showed that none of the light regimes: continuous darkness, continuous light or alternating light and darkness, had any marked difference in the amount of conidia that germinated (Tab. 2). There was a decrease in % germination with increase in fungicide concentration, 0-500 ppm of benlate (butyl carbamoyl - 1 benzimidozolyl - 2) and 0-100 ppm of brestan (fentin acetate + maneb) (Tab. 3). At 1000 ppm of benlate and at 250-1000 ppm of brestan, no germination was recorded.

DISCUSSION

The process of germination of conidia of *Cercospora cruenta* was slow on glass slide but fast on cowpea leaf decoction nutrient agar and host epidermal strips. This observation may be due to the absence of stimulatory exsudates or nutrients on the glass as a substrate. The period before germination commences depends on the presence of substrates to produce critical amount of energy for the transformation of spore cells into germ tubes (Gottlieb, 1964). This could explain therefore the fast germination recorded on the culture medium and the host epidermal strips. Furthermore, the non-production of hyphae on glass slide would be as a result of non-availability of nutrients to keep germ tube growing.

Table 3 - The effect of different concentrations of benlate and brestan on the germination of conidia of *C. cruenta* on cowpea leaf decoction nutrient agar incubated for 6h at 28.5°C.

Tableau 3 - Effet des différentes concentrations de benlate et de brestan sur la germination des conidies de *Cercospora cruenta*, sur gélose nutritive à base de décoction de feuilles de *Vigna unguiculata*. Incubation 6h à 28,5°C.

fungicide conc. (ppm)	% mean germination	
	benlate	brestan
0	92.8 ± 1.9	89.0 ± 1.3
10	81.6 ± 1.5	69.8 ± 1.2
50	75.5 ± 1.0	60.6 ± 1.0
100	72.0 ± 1.0	34.0 ± 0.7
250	53.5 ± 0.7	0
500	9.7 ± 0.1	0
1000	0	0

Data are means of 5 replicates.

The germination of the conidia on any of the 3 substrates did not show the formation of secondary conidia or appressoria. However, the production of 10 germ tubes from 10 cells of a conidium on the medium during this study may be significant. The rich nutritional composition of this medium might have encouraged the massive production of germ tubes. Maximum

conidial germination was recorded at 16.10^4 spore/ml while at 24.10^4 spore/ml, few germination occurred.

Spores of some fungi show self inhibition and they do not germinate if too densely crowded (Allen, 1955; Tarr, 1972). Musumeci & al. (1974) also observed this crowding effect in coffee rust where increasing concentration of uredospore almost completely suppressed germination.

The temperature range for the germination of conidia of *C. cruenta* obtained during this study is 15-40°C with optimum at 30°C. Berger & Hanson (1963) obtained in *C. zebrina* a germination range of 8-36°C with optimum between 20-30°C and this same range was obtained for *C. arachicola* (Oso, 1972). *C. cruenta* could not germinate at 5°C and this would mean that inactivation of conidia could occur at low temperature (0-10°C).

There was no conidial germination at relative humidity levels below 32.5% in *C. apii* and *C. contraria* (Emua, 1980) while in *C. arachidicola* at 88% RH germination of conidia was not recorded (Oso, 1972). Conidial germination was recorded at 32.5-100% RH in *C. cruenta* during the present study. The pathogen therefore cannot germinate and cause infection under dry environments. The germination of conidia under different light regimes did not show any marked significant difference but there was a gradual decrease in germination with increase in light intensity. This effect could be due to variation in temperature which was recorded and observed with increase in light intensity. However, heaviest sporulation of *C. cruenta* under both *in vitro* and *"in vivo"* conditions occurred during the period of continuous darkness. Alasoadura & Fajola (1970) recorded optimum conidial production in culture in *C. nicotianae* in continuous darkness at 89% RH. Heaviest conidiation was also reported in five *Cercospora* spp. (Fajola, 1978) in continuous darkness. The process of conidiation therefore is more enhanced at night hence it is not surprising to commonly observe heavy aggregation of conidia and conidiophores of *Cercospora* spp. in the early hours of the day with dew.

The *C. cruenta* leaf spot disease of cowpea is not common during the dry season with intermitent rain but appears at the begining of the rainy season. Therefore the results of this investigation have provided some information on the optimal environmental conditions viz: 80-100% RH, 20-30°C temperature range that would favour its conidial germination and sporulation. These are 2 of the factors necessary for initiation and subsequent spread of infection. The results obtained from the effects of fungicides on spore germination show that brestan was more effective than benlate in the inhibition of conidia of *C. cruenta*. Brestan would therefore control this pathogen more efficiently on the field.

Non seulement l'Auteur a réussi l'établissement de clés très pratiques, mais il a également fait oeuvre utile en apportant éclaircissements et indications sur des problèmes de Mycologie que bien des livres ne traitent que succinctement ou pas du tout. On doit lui savoir gré d'avoir mis rapidement à la disposition de tous un document de travail basé sur ses notes personnelles. Une telle hâte transparaît évidemment dans le style - de plus, souvent emphatique - qui n'a pas été assez travaillé et dans l'illustration, expressive, mais parfois peu soignée. Ces quelques réserves ne portent cependant qu'une ombre légère aux qualités certaines d'un ouvrage qui fut le premier en France à suivre les recommandations du Code International de Nomenclature Botanique; la preuve en est qu'il a remporté et remporte encore, avec son troisième tirage, un large succès.

J. Perreau

PARMASTO E. & PARMASTO I., 1987 - Variation of basidiospores in Hymenomycetes and its significance to their taxonomy. Berlin, J. Cramer, *Bibliotheca Mycologica*. Band 115, 168p., 1 fig., 36 tabl. (appendix by T. Möls).

Il est reconnu depuis longtemps que les caractéristiques des basidiospores présentent une valeur certaine en taxinomie, notamment dans le domaine de la spécification. Mais cette importance trouve ses limites du fait de la grande variabilité des particularités sporales. A l'aide des travaux déjà publiés sur le sujet et de leurs observations personnelles, les Auteurs analysent tous les aspects et enseignements à tirer de la variation de la longueur et de la largeur chez les éléments de dissémination produits par les basides des Hyménomycètes.

Ainsi que l'indique une table des matières très détaillée, la première partie de l'ouvrage expose d'abord la méthodologie suivie, avec naturellement une insistance spéciale sur les procédés de mesure. Puis une revue des multiples facteurs qui peuvent modifier la taille des spores, montre la complexité du problème. Les résultats, étayés par de nombreuses données numériques et interprétés en distinguant toujours l'individu ou l'espèce, conduisent à l'appréciation biologique des variations et leurs possibilités d'application en taxinomie. Enfin des informations apportées sur ce même phénomène de variabilité sporale dans des groupes tels que Péronosporales, Erysiphales et Urédinales, permettent d'établir des comparaisons intéressantes avec les conclusions avancées pour les Hyménomycètes.

Ce livre qui, d'un côté, disèque littéralement une question aussi diverse qu'essentielle dans une partie de la Mycologie et, par ailleurs, en propose une évaluation raisonnable, constitue une documentation indispensable au sporologue comme au taxinomiste.

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